TECHNICAL REPORT

Application of Elemental Fingerprinting to Evaluate the Dynamics of Larval Exchange

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Abstract

Most marine invertebrates have planktonic larval stages that act as agents for increased dispersal and gene flow between adult populations, but little is known about the spatial scale and strength of connectivity that results from larval dispersal. The primary objective of this research was the development of trace elemental fingerprinting methods to assess patterns of exchange among mytilid mussel populations in southern California. We also developed a model to predict passive larval exchange by currents in order to draw comparisons with realized dispersal. A major project result was validation (for mussels) of the premise that site-specific water mass differences in elemental composition are imparted to carbonate shells of larvae when the shell is deposited. Thus, the larval shell contains a chemical record of its past and is retained by newly recruited mussels as they grow. Accomplishments include (1) development of solution based- and Laser Ablation-Inductively Coupled Plasma Mass Spectrometry (LA-ICPMS) analytical protocols to identify detectable trace elements in bivalve shells < 2 mm, (2) development of rearing and outplanting techniques to validate larval shell signatures, (3) quantification and comparison of site-specific trace element composition in distinct regions of bivalve shells, including larval prodissoconch and juvenile shells, and (4) classification of elemental signatures of individual shells to distinguish larval origins using discriminant function analysis (DFA). Sampling over 2 years, quarterly and at 1-week intervals revealed elemental signal variation in time, and indicated the need to validate signals and conduct connectivity analyses in the same time period. Although we have yet to confirm patterns of connectivity, significant progress has been made in the novel application of elemental fingerprinting to marine invertebrates. Ultimately this approach will allow us to

incorporate dispersal information into population dynamics models to better understand the consequences of different patterns of population connectivity.

Background

Many marine benthic invertebrates have a planktonic larvae that acts as an agent of dispersal and gene flow between adult populations (Thorson 1950, Grahame & Branch 1985). Knowing dispersal trajectories and fates of planktonic larvae are essential to understanding species' population dynamics (Eckman 1996) and for effective conservation. The degree to which populations within a metapopulation exchange individuals (larvae) and by inference, the extent to which they seed themselves, has been termed connectivity (e.g., Moilanen & Nieminen 2002). Processes such as ocean circulation, duration of the planktonic period, larval behavior, and settlement success will determine spatial and temporal patterns of population connectivity. Patterns and strengths of connectivity are important for predicting population responses to environmental change. Effective management of marine resources requires knowledge of which sites or habitats supply successful recruits. Conservation of a population (e.g., through establishment of reserve networks or no-catch zones) requires that one also guarantees a supply of young, ideally by conserving the source of those young.

Determining dispersal trajectories of marine invertebrate larvae remains one of the most difficult problems in biological oceanography because of their small size and high dilution rates. Approaches to this problem have included visual tracking, analysis of distribution in relation to isolated sources or physical structures, use of lagrangian mimics, modeling (hydrodynamic, behavior, and energetic) or use of tags (reviewed by Levin 1990, Thorrold et al. 2002). Although artificial tagging has been attempted, dilution of tagged individuals *in situ* has limited the usefulness of this approach (e.g., Levin 1993, Anastasia et al. 1998). Methods that take advantage of inherent genetic variation at the population level, including allozyme, mtDNA, and hypervariable nuclear DNA markers, can make a powerful case for complete isolation (Helberg et al. 2002). However, these techniques are limited in their ability to quantify connectivity and gene flow among populations on ecological time scales. Tags that occur naturally, label all larvae within a site, and carry no history of past migrations, provide a more tractable option.

The trace elemental and isotopic composition of carbonate structures in living organisms provide such a tag. Carbonate skeleton, tests, shell, statoliths or otoliths reflect subtle differences in chemical and physical conditions of the environment. This approach has been exploited in coral skeletons (e.g., Mitsuguchi et al. 1996), deep-sea gastropods (Killingley & Rex 1985), ostracod shells (reviewed in DeDeckker et al. 1988) and foraminiferan tests (e.g., Rosenthal et al. 1997) to generate historical climate and productivity records. Recently, elemental fingerprinting techniques have been applied to otoliths (ear bones) in a variety of fish larvae to identify larval dispersal trajectories and retention patterns (Swearer et al. 1999), rearing sites (Gillanders & Kingford 1996, Kennedy et al. 1997, Yamashita et al. 2000), stock mixing (Edmonds et al. 1989, 1991, Campana et al. 1994), and natal site fidelity (Campana & Gagne 1995, Secor et al. 1995,

Thorrold et al. 2001). Comparable methods have only recently been applied to dispersal problems in invertebrates. DiBacco and colleagues pioneered these efforts with crustacean larvae using zoeal elemental composition to document significant exchange of larvae between San Diego Bay and the coastal zone (DiBacco & Levin 2000, DiBacco & Chadwick 2001). Crustacean larvae, however, do not retain hard parts into adulthood, so these studies were limited to relatively short time scales. Many mollusks, on the other hand, retain their larval shell as part of the adult structure and are well suited to methods that reconstruct larval trajectories from elemental analysis of settled animals. Differences in element concentrations between and within coastal and bay habitats due to differences in temperature, anthropogenic inputs, geological processes (e.g., weathering), or riverine inputs are reflected in organisms inhabiting such sites.

Approach and Methods

Elemental "fingerprinting" utilizes a naturally induced tag that incorporates specific environmental signals such as trace elements, salinity or temperature, to track movements of larvae. It is based on the premise that site-specific water mass differences in elemental composition are imparted to aragonitic shells of larvae when they are deposited. This shell, which contains a chemical record of its past, is retained by newly recruited mussels as they grow. By examining the larval shell on new mussel recruits we can reconstruct their origins and possibly their trajectories. We have adopted 3 means to validate the method: (1) analysis of shell composition for larvae spawned in the lab and out-planted at known sites (2) characterization of the composition of shell material freshly deposited by new recruits and (3) quantification of seawater composition at various locations.

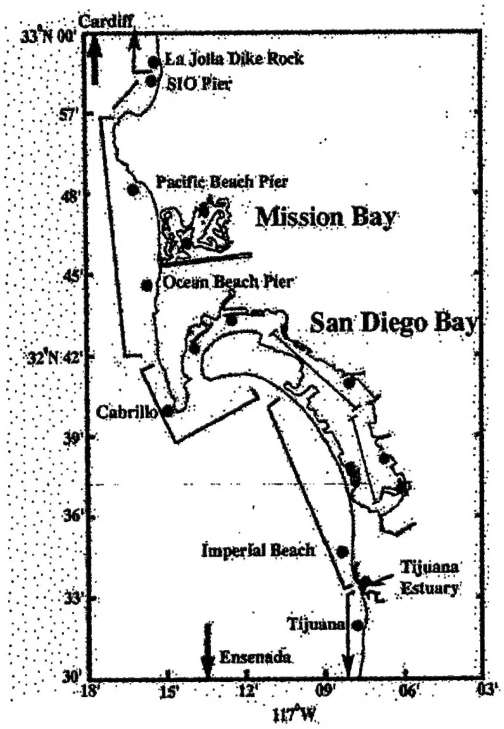

Figure 1. Map of southern California mussel collecting sites including San Diego Bay (n=5), Mission Bay (3), Tijuana Estuary (1) and exposed coastal habitats (10). Not shown are Cardiff to the north and Ensenada to the south. Probable natal zones are indicated as lines on the map. Within these regions, Prodissoconch I shell elemental signatures are likely to be indistinguishable.

For this study, we collected newly settled, post-larval recruits in San Diego Bay, neighboring embayments, and surrounding nearshore coastal habitats. Sampling was conducted quarterly at 14 sites (Fig. 1) and weekly for several months at the Scripps Pier and in San Diego Bay. Molecular PCR techniques were developed to distinguish newly settled *Mytilus californianus* from *M. galloprovincialis* using soft tissues. The elemental composition of larval and post settlement shell in recruits < 1 mm was determined first in solution, initially with an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES), then with an ICP - Mass Spectrometer (ICP-MS). Subsequently we made a technological transition from ICP-MS solution-based

analysis to Laser Ablation-ICP-MS solid phase analyses, with the assistance of a DURIP award to purchase the laser ablation unit. Elemental composition of larval and post settlement shells were determined for new recruits. Discriminant Function Analysis (DFA) was used to determine canonical variables (linear combinations of sampled trace elements) that serve as 'fingerprints' to identify the locations where larvae developed.

To examine the potential effects of passive transport, a predictive model of connectivity between different larval source habitats was constructed for the San Diego region (Fig. 2A.). Exchange rates between modules were estimated based on results of field studies in San Diego Bay, Mission Bay and coastal waters. Box sizes were scaled according to typical excursion lengths of the exchange flows (e.g., tidal excursion lengths in the bays with internal mixing of box every tidal cycle).

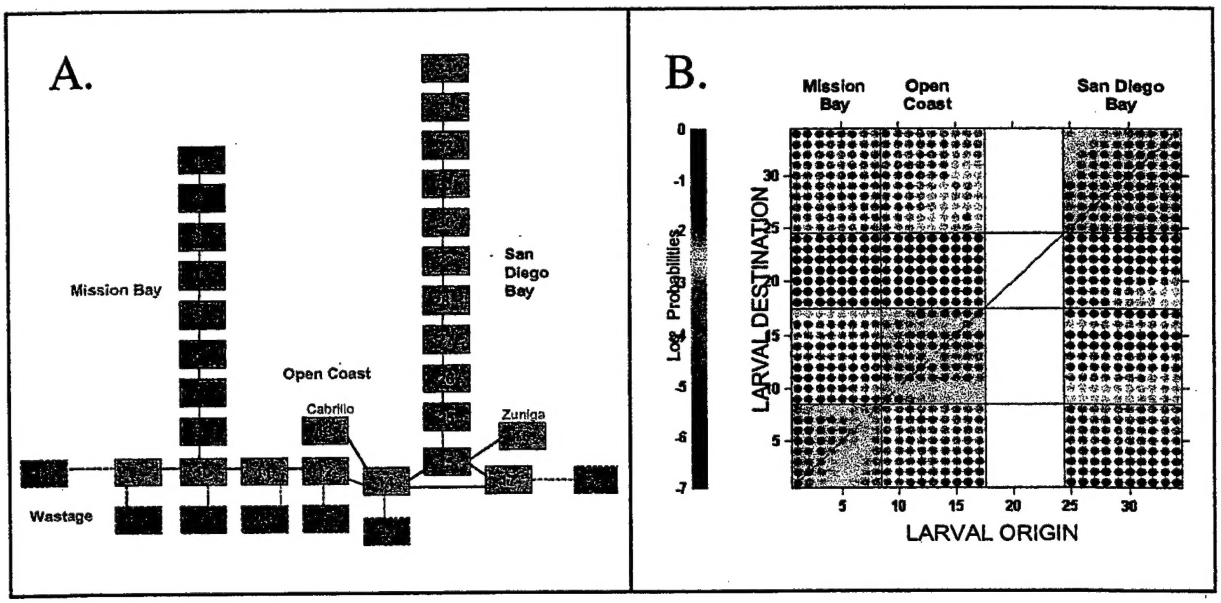

Figure 2 A. Schematic layout of boxes representing Mission Bay, San Diego Bay and coastal waters in the connectivity box model. Boxes have real volumes. Advective and diffusive exchange rates between boxes is estimated from field data. Connectivity between boxes is calculated by releasing larvae in one box and determining what percentage is found in each box at the end of the larval period; B. Color coded plot of connectivity matrix (probability of larva released at origin box X being in destination box Y at end of larval period). This base case is for passive larvae with a 30-day planktonic period, no behavior, zero mortality, and zero mean flow. No larvae are released in "wastage" boxes 18 to 24 (no spawning habitat). For small-volume boxes (e.g., 1 and 34), the final larval concentration may be high, but the probability of that box as a destination is low as the box volume is small. Strength of settlement is more closely related to larval concentration (connectivity/volume).

Results

Rearing and Identification

Key to validation of the fingerprinting techniques is the ability to (1) culture and rear larvae in the laboratory and in the field, and (2) distinguish *M. californianus* from *M.galloprovincialis* as newly settled recruits. We successfully reared *M. californianus* from spawn until settlement in the lab. Larval outplanting techniques were tested to acquire larval shell signatures from our collection sites. Successful outplanting of *M. californianus* and *M. galloprovincialis* was achieved by raising them *in situ* for 1 week in PVC 'larval homes' (Fig. 3). In order to identify new recruits, graduate student B. Becker has collaborated with SIO professor Ron Burton to create primers that distinguish DNA in *M. californianus* from that of *M. galloprovincialis*. This is essential for distinction of the two mytilid species in open coast environments where they both can settle.

Figure 3. Larval home made of PVC tubing.

Trace elemental analyses

Water samples, collected from three sites in San Diego Bay, CA, two sites inside Mission Bay, CA, and eight sites along the open coast of southern California have been analyzed using solution based ICP-MS with only filtration, acidification and dilution necessary to prepare samples. Among the 17 elements examined, concentrations of Ba, Cd, Cr, Ca, Sr, Mn, Mg and Li, along with temperature and salinity, were useful in discriminating locations (Fig. 4A).

Trace element analyses by ICP-OES and subsequent discriminant function analyses have indicated our ability to distinguish mussel shells based on site, species, and size. Small (<250,000 ppb Ca) *Mytilus* individuals could be distinguished from Mission Bay, San Diego Bay and the open coast (Fig.4B). Among *Mytilus* recruits, concentrations of Ag, Cd, Cu, Fe, Ni, Pb, Sr, and Zn were elevated in samples from Dana Landing in Mission Bay and in most cases, from Harbor Island in San Diego Bay relative to open coast sites.

Elements such as Mn and Mg did not show a difference among sites for *Mytilus* spp. Within the outer portion of San Diego Bay, Harbor Island mussels were distinct from those on Shelter Island and Coronado Tidelands, mainly due to elevated Al, Ba, and Cu concentrations. Among open coast sites, Crystal Pier (Pacific Beach) mussels were distinct, driven by Mn, Al, Cd and Sr, but this could be due to the fact that these animals were considerably smaller than the others.

Mytilus species were found to be distinct from *Musculista senhousia* (Fig. 4C), largely due to differences in Cd, Ba, Sr, Mg and Mn, To confirm species identifications, *Mytilus* soft parts are being identified by molecular (PCR) methods while shells are analyzed for trace element composition.

Mussel shell size, inferred by Ca content, was related to elemental concentration. High concentrations of trace elements were found only in the smallest *Mytilus spp.* for all elements examined except those known to be sensitive to temperature and salinity (Mg, Sr), and except for Zn and Mn, for which the pattern is more ambiguous. Similar size-trace element relationships were observed in *Mytilus* spp. and *Musculista senhousia.* Size alone is able to discriminate among mussel shell compositions from a single site. For example, differences in Al, Fe, Sr, Cd, and Mn drive size-related variation in *Mytilus* from the Scripps Pier (Fig. 4D).

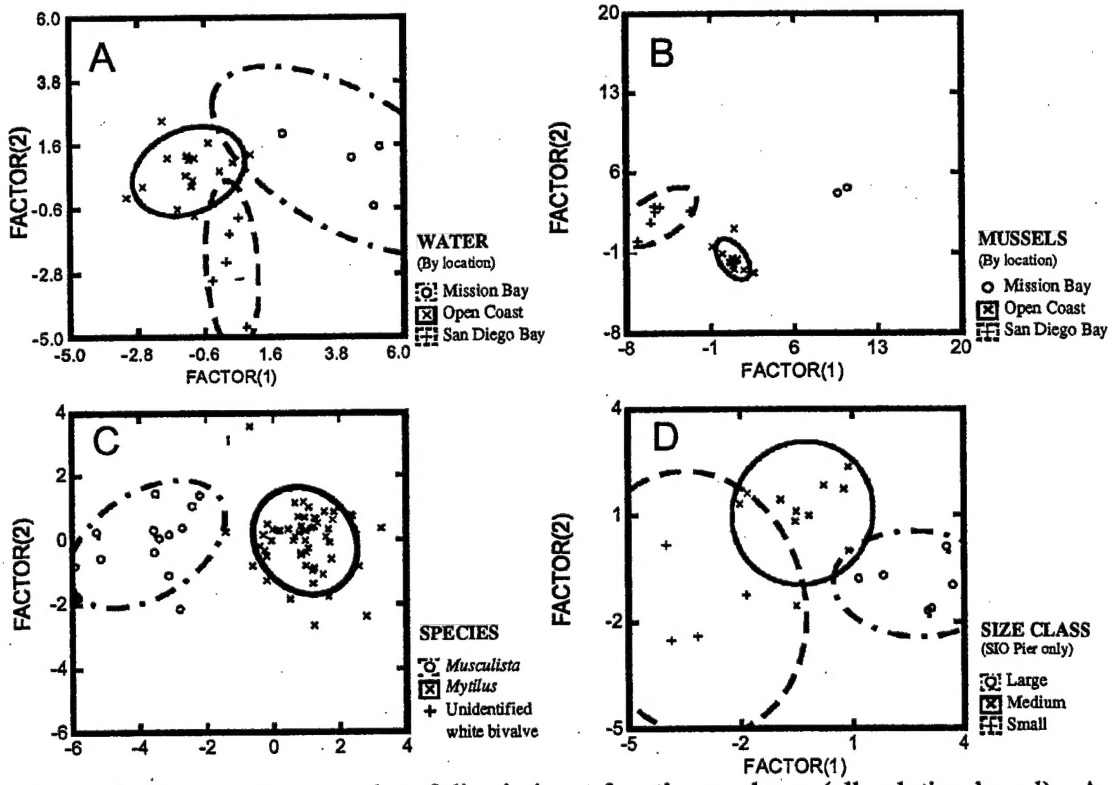

Figure 4. Canonical scores plot of discriminant function analyses (all solution-based). A. Seawater analysis grouped by location (includes temperature and salinity), summer 2001; B. Analysis of Mytilus, grouped by location, Summer 2000, Winter 2001; C. Analysis of mussels grouped by species; D. Analysis of Scripps Pier Mytilus, grouped by size class (inferred from Calcium values)

Solution based elemental techniques limited our ability to target only the larval portion of bivalve shells (about 200 _m diameter) or to distinguish larval versus newly settled juvenile shell. Acquisition of a 213 nm Laser Ablater combined with a double focusing, single collector, magnetic sector ICP-MS permitted elemental analysis of specific regions of the shell (20-100 _m diameter) to be analyzed on individuals of varied ages.

To carry out solid phase analysis of mussel shells using LA ICP-MS, we developed methods for cleaning and mounting shells, tested instrument settings such as laser intensity, form (spot/raster) and duration and refined computer data collection techniques. We explored both spatial and temporal variation in elemental shell composition, obtaining information necessary to implement elemental fingerprinting studies of mussel dispersal.

Laser ablation data for *M. californianus* recruits revealed that elemental signatures could be measured for a suite of elements (Sr, Pb, Cd, Zn, Co, Al, Ag, Mg) for components of the larval shell ca. 20-80 µm in diameter (Fig. 5; 2 elements are given as an example). Elemental signatures determined at this spatial resolution allow elemental analysis of specific components of the larval shell representing various phases of planktonic development.

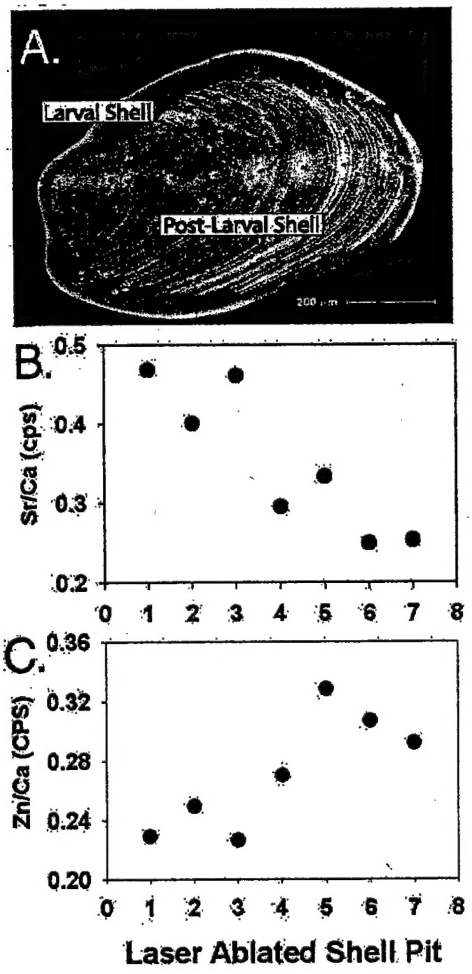

Figure 5. Fine-scale trace element analysis of a recently settled *M. californianus* shell. Numbering on the abscissa of panels B & C corespond to laser ablated holes in the larval shell (1-3) and post settlement juvenile (4-7) shell regions.

Elemental signatures (all expressed as ratios to calcium) were determined using LA ICP-MS for the larval and post-larval shell of recently recruited *M. californianus* (< 3 mm) in southern California. Discriminant function analyses (DFA) of post settlement shell trace elemental concentrations effectively distinguished mussels collected from open coast sites (Cabrillo, Pacific Beach, Ocean Beach, La Jolla and Cardiff) along a 35-km stretch based largely on Cr, Zn, Sr, and U (classification success 88%)

(Fig.6A). These data suggest that variation in elemental composition is not directly linked to distance among sites. Recruit shells from within Mission Bay (MB) and San Diego Bay (SDB) could be distinguished from open coast recruits based on Pb, Cr, Sr and Ba (82% success).

Quarterly samples from the Scripps Pier (La Jolla) reflect temporal variability, such that the SIO pier recruit shells were distinct from samples to the north (La Jolla Dike Rock and Cardiff) during winter and spring, but not during fall (Fig. 6B). Such variation indicates that that recruit sampling and validation work are best conducted during the same time period. DFA of the corresponding larval shells (origin unknown) revealed distinct signatures for two bays and 3 open coast site groupings (Fig. 6C). The similarity of larval shell composition at adjacent open coast sites (Fig. 6C) may indicate a similar origin for those recruits. These results indicate that site-specific elemental signatures can be detected among *Mytilus* shells. However, to provide the data necessary to assign larval origins based on elemental composition of larval shell we must know the site-specific larval signatures. Methods for validation by larval outplanting (*in situ* rearing) have been developed and *Mytilus* larvae have been reared at selected sites and recovered successful. Additional deployments and elemental analyses are continuing.

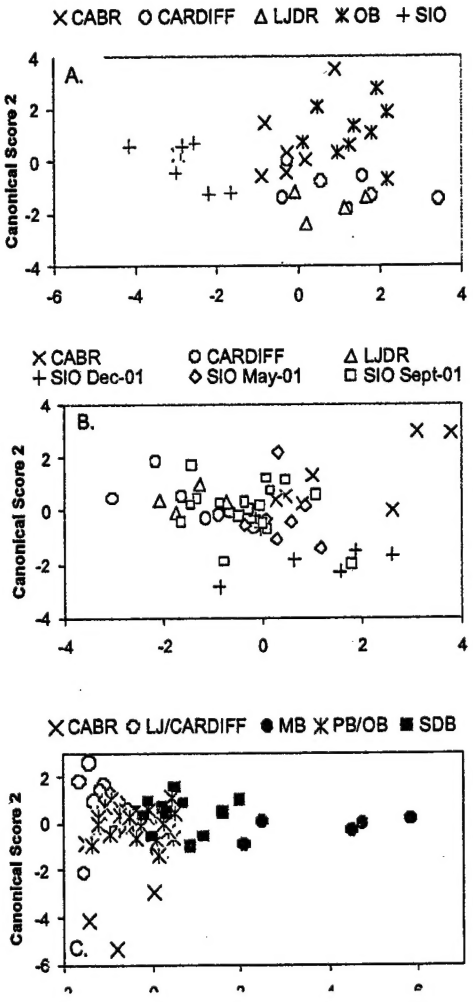

Figure 6. Canonical DFA based on elemental composition of *M. californianus* (A) post settlement shells from known open coast locations, collected Dec. 2001, (B) temporal variation in post set shells during May, Sept., Dec. 2001 at the SIO pier in relation to other open coast sites (Dec. 2001), (C) *Mytilus* larval shells of unknown origin sampled from recruits to Bay (MB, SDB) and opencoast locations (Cabrillo, LJ/Cardiff and PB/OB). Within-site variation may indicate larvae of different origins.

Fine-scale temporal variation in trace elemental composition of new recruit shell edge was examined by sampling weekly at a single site (SIO Pier) during 4 weeks in Feb. 2002. We noted instability in elemental composition for Cr (P=0.006), Ag (P=0.021), and Cd (P=0.022) (Kruskall Wallace test), and no significant change for

Pb, Mg and Mn. Recruitment variation, measured as settling mussels per g byssal thread, was noted among sampling dates. The sampling date with 3-4x higher recruitment than the other weeks yielded settlers with lower Cr, Ag, Mg and Cd concentrations, perhaps indicating input of oceanic or upwelled water (Fig. 7). Multiple analyses of the same shells on different days revealed no machine drift, analysis date or firing error effects for Mg, Mn, Cr, Ag, and Pb ($P>0.05$ Wilcoxon signed rank test). For elemental fingerprinting to aid interpretation of dispersal, either temporal variation within each site must be lower than that among sites, or signatures must be validated during the period when larvae are developing (ideally both should occur).

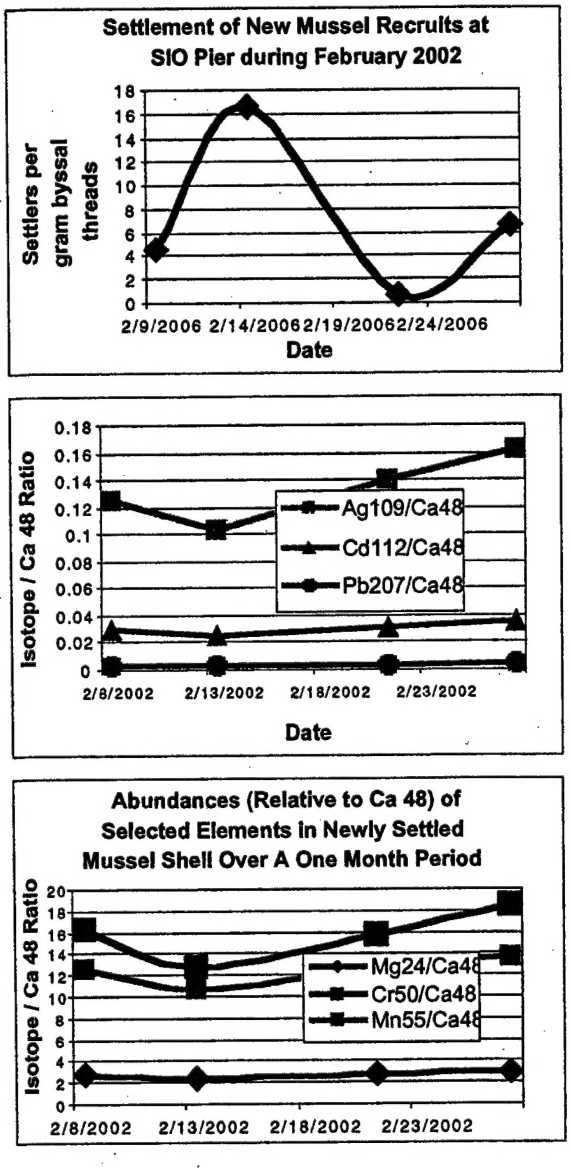

Figure 7. Weekly variation in recruitment and shell edge elemental composition during February 2002.

Connectivity Model

Connectivity results are presented for an initial case of mussel larvae with 30-day passive dispersal, with no mortality or mean flow (e.g., no river inflow to bays and no alongshore mean coastal current). Connectivity is shown in Fig. 2B, with connectivity defined as the

probability that larvae released in box X are found in box Y after 30 days (for a given origin, the sum of values is one).

For this scenario (no behavior), model results suggest that a significant portion of larvae spawned in Mission Bay (about 10% released from the back of the bay) and in San Diego Bay (about 50% released from the back of the bay) would be retained within their bays. Of the remainder, a relatively large fraction are wasted (leave the area of interest), and for San Diego Bay, some will accumulate outside the Bay mouth. Most larvae released from populations along the outer coast are lost. The remainder of coastal spawned larvae are distributed along the shore; few enter the bays. Sensitivity analyses and estimates of uncertainty are underway. Other model runs include mean flows (advection), non-uniform spawning, non-uniform mortality, and exchange rates that are adjusted to account for larger tides and or estuarine circulation effects.

Population modeling

Stage-structured matrix models were constructed for the crab *Pachygrapsus crassipes* to explore population-level consequences of transport to and development in bay versus coastal waters of southern California. A 4-stage model (zoea 1, zoea 2-6, megalopae, post settlement stage) yielded highest population growth rates for larvae that were spawned and developed along the open coast, lowest rates for larvae spawned and developed in SDB, and intermediate/similar values for larvae spawned in one place and developed in the other. Elasticities suggest that population growth rate is most sensitive to changes in fecundity and larval growth rates. C. DiBacco's participation in an NCEAS workshop led to a pilot dispersal-population model linking an invasion kernel (bay source entering coastal waters) to population matrix modes.

Project Implications

This research will advance understanding of marine invertebrate dynamics by (a) adapting laser ablation techniques to the study of bivalve larval origins and exchange, (b) relating physical exchange probabilities to actual estimates of bay-ocean and bay-bay larval exchange, and (c) providing practical application of connectivity information to the management of mussel populations in protected areas (e.g., Cabrillo Nat'l Monument). Expansion of element-based tagging approaches to identification of invertebrate recruit origins, and to questions of population connectivity should open up a wide range of applications. These include assessment of the interdependence of different habitats, evaluation of spatial and temporal variability in recruit dynamics, and determination of pollution consequences.

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14. ABSTRACT

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15. SUBJECT TERMS
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